IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Roberts *et al.*

Serial No.: 10/601,841 Docket No.: 032026-0731

Filed: June 23, 2003 Examiner: M. Perreira

For: **SYNTHESIS OF ^{17}F LABELED FLUOROALKANES**

DECLARATION OF ROBERT J. NICKLES

I, Robert J. Nickles (Jerry Nickles), do hereby declare and state as follows:

I am a professor of medical physics, radiology and physics at the University of Wisconsin-Madison (UW-Madison). I have held my current position since 1981.

I received a Bachelors of Science in Math and Physics from UW-Madison in 1962, a Masters in Science in Nuclear Physics from the University of Sao Paulo in 1967, and a Ph.D. in Nuclear Physics from UW-Madison in 1968.

A copy of my professional biography is attached.

I am a coinventor of the subject matter claimed in the above-referenced patent application.

I have reviewed the disclosures of Ruth et al., Synthesis of C-11 and F-18 Labelled Compounds for Biomedical Applications: Current Status and Challenges for the Future in the Journal of Radioanalytical and Nuclear Chemistry, Vol. 203, No. 2, pages 457-469 (1996) (hereinafter "Ruth") and Pike et al., Novel Use of an Isotope Separator to Determine the Position of Fluorine-18 in Labelled 1,1,1,2-Tetrafluoroethanes in Organic Mass Spectrometry, Vol. 29, pages 499-504 (1994) (hereinafter "Pike").

On pages 462-465 Ruth describes electrophilic and nucleophilic fluorination reactions used in ^{18}F labeling. On page 500 Pike describes nucleophilic fluorination reactions used in ^{18}F labeling 1,1,1,2-tetrafluoroethane. Neither the electrophilic reactions described in Ruth nor the nucleophilic reactions described in Ruth and Pike could be used to incorporate ^{17}F into organic molecules to produce a radiopharmaceutical for imaging use.

Conventional electrophilic reactions for ^{18}F labeling organic molecules, including the electrophilic reactions described in Ruth, could not be used to produce an ^{17}F labeled radiopharmaceutical for imaging use because they involve reacting (bubbling) the gas phase $^{18}F_2$, diluted in an inert gas with a precursor, generally a mercurated or stannylated compound, dissolved in a suitable solvent such as chlorofluoromethane. After the electrophilic de-metallation, the subsequent steps involve flash chromatography to remove the alkyl-stannyl

group, evaporation of the organic solvent, hydrolysis, and HPLC clean-up prior to the final radiopharmaceutical steps. These bubbling, alkyl-stannyl removal, hydrolysis, and HPLC steps generally require times of the order of tens of minutes, typically 40 minutes to make ^{18}F-fluoro-DOPA. This time scale is completely impractical with ^{17}F, with a 64 second half life.

Conventional nucleophilic reactions for ^{18}F labeling organic molecules, including the nucleophilic reactions described in Ruth and Pike, could not be used to produce an ^{17}F labeled radiopharmaceutical for imaging use because of the same unfavorable time scale. Here, the situation is even worse, with standard nucleophilic labeling conditions starting with aqueous ^{18}F-fluoride, separated from the ^{18}O-enriched target water by a trap and release column. The ^{18}F-activity is then supported by a phase transfer catalyst such as Kryptofix 2.2.2 / K^+ in acetonitrile / water, which is then azeotropically distilled to finally reveal the supported ^{18}F activity in an organic solvent (e.g. acetonitrile) with rigourous exclusion of water. The nucleophilic attack can then proceed with the chosen precursor material, followed by solid phase (Sep-pak) extraction, hydrolysis and deprotection, for final clean-up. These steps, particularly the removal of all traces of water from the Kryptofix/K^+F^- require tens of minutes under gentle conditions, totally impractical for the 64-second half life of ^{17}F. For these reasons, the fast, flow-through ("jet") chemistry that has been developed for our production of the ^{17}F-fluoroalkanes, as described in the above-referenced patent application, is unique and is not discussed in the cited literature.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Nov 13, 2006 Signature: Robert J. Nickles

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Professor of Medical Physics, Radiology, and Physics.

B.S. in Math/Physics from the University of Wisconsin-Madison, 1962.
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Professional Experience
1968-1969 Postdoctoral Fellow, Nuclear Physics, University of Wisconsin, Madison
1969-1972 James A. Picker Fellow, Niels B?Institute, Copenhagen, Denmark
1972-1976 Assistant Professor of Radiology, University of Wisconsin, Madison
1976-1981 Research Career Development Award
1976 Visiting Professor, University of Groningen, Netherlands
1976-1981 Associate Professor of Radiology, University of Wisconsin, Madison
1977 Visiting Professor, University of Sao Paulo, Brazil
1980 Exchange Visiting Professor, University of Helsinki, Finland
1981-present Professor of Medical Physics and Radiology, University of Wisconsin-Madison
1991 Exchange Visiting Professor, Paul Scherrer Institut, Villigen, Switzerland

Research Interests
My research interests have focused on the production and application of positron emitters in radionuclide imaging. While historically PET is linked to the four major radioisotopes ^{11}C, ^{13}N, ^{15}O and ^{18}F, the work at Wisconsin have broadened this list to dozens of new candidates, opening up unique possibilities for labeling agents with

- very short-lived tracers, such as ^{10}C, ^{14}O and ^{17}F for tracking blood flow
- transition metals (Mn, Cu,..) well-suited for chelation chemistry
- positron-emitting ^{94m}Tc to bridge the gap between PET and SPECT
- and scaled up production to satisfy diverse needs, such as studies with radioactive ion beams.

The application of these agents centers on the integrated resources of the UW Medical Physics Dept, including the first prototype CTI 11 MeV proton cyclotron, serving a fully equipped radiochemistry lab, feeding a CTI 933/04 ECAT scanner dedicated to basic imaging research. Instrument development underpinning these facilities has been an integral part of the dissertation projects of 15 PhD candidates graduating from the cyclotron group, and as many excellent MS students.

Recent publications

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